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Novel Diamidines with Activity against *Babesia divergens In Vitro* and *Babesia microti In Vivo*▽

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Dicationic diamidines, such as diminazene and pentamidine, are well-studied chemotherapeutic agents with significant activity against parasitic diseases. The *in vitro* activities of novel diamidine compounds against the *Babesia divergens* strains 1903B and 4201 were investigated. The most potent compound, a diphenyl furan, had a 50% inhibitory concentration (IC_{50}) of 1.5 ng/ml. In a murine model, several test compounds were effective enough to cure mice infected with *Babesia microti* at a dose of 12.5 and/or 25 mg/kg of body weight given by the subcutaneous route for 4 days. The best antibabesial properties were exhibited by terphenyls, benzimidazoles, diphenyl furans, pentamidine, and pentamidine analogues.

Babesiosis is a tick-borne infection caused by intraerythrocytic protozoan parasites of the genus *Babesia* and affects wild and domestic animals worldwide. It is a well-known disease of veterinary importance in cattle, horses, and dogs which causes considerable economic losses in livestock industry and is gaining interest as an emerging zoonosis. Bovine disease is most common in tropical and subtropical regions (*Babesia bovis*, *Babesia bigemina*), but infections are also seen in Europe (*Babesia divergens*, *Babesia major*). Clinical manifestations like fever, malaise, hemolytic anemia, and hemoglobinuria may be absent, but babesiosis can develop into a severe, rapidly fatal disease. Even though a number of effective antibabesial drugs exist, they are not readily available in all areas due to safety, residue, or marketing issues. The most widely used babesiacides are imidocarb dipropionate and diminazene aceturate; however, only imidocarb is able to consistently clear the host of parasites and has chemoprophylactic properties (29). Thus, concerns about development of resistance are growing, and the need for alternative compounds for the veterinary market is evident.

Human infections with *Babesia* species have been known since the late 1950s. While in Europe the causative agent in cases of babesiosis in splenectomized individuals was identified as the cattle species *B. divergens*, the rodent species *Babesia microti* was reported in most cases of human babesiosis in North America (8, 14, 28), where the disease is endemic and transmitted by the tick *Ixodes damiani* (also known as *I. scapularis*). *Babesia duncani* and *B. divergens*-like organisms later attracted attention in the west and midwest of the United States. Sporadic cases of human babesiosis have also been identified in Asia, Africa, and South America (28).

Diamidines have a long history as chemotherapeutic agents against protozoan infections, and their synthetic producibility, as well as their low molecular weight, makes them an attractive drug class. The activity is due mostly to the selective accumulation by the parasite rather than the host cell. Aromatic dicationic molecules are thought to act by binding to the minor groove of DNA at AT-rich sites, which are present in many parasitic organisms, and thus they may possibly inhibit DNA-dependent enzymes or cause direct inhibition of transcription (26). In previous studies, the significant antiparasitic activity of novel diamidines against *Trypanosoma brucei* and *Plasmodium* spp. has been shown (2, 15, 25, 26).

In the present study, we selected new diamidine compounds according to their *in vitro* activities against *Plasmodium falciparum* and demonstrated their *in vitro* potential against the two *B. divergens* strains 1903B and 4201. Subsequently, a selection of compounds was evaluated in a *B. microti* mouse model.

MATERIALS AND METHODS

Parasite strains and cultivation. The two bovine *B. divergens* strains 1903B and 4201 were kindly provided by Laurence Malandrin of the Ecole Nationale Vétérinaire de Nantes, Nantes, France. Continuous *in vitro* cultures were maintained in human red blood cells (RBC) diluted to 5% hematocrit in RPMI 1640 with 25 mM HEPES and 2 mM glutamine (BioConcept, Allschwil, Switzerland) supplemented with 5 g/liter Albumax I (Gibco/BRL Life Technologies, Belgium) and 10 μg/liter gentamicin (Sigma, Steinheim, Germany). All cultures were kept in 25-ml flasks at 37°C in a 4% CO_2, 3% O_2, 93% N_2 gas mix. The medium was changed daily, and subpassages were performed every 2 to 3 days when the parasitemia reached 20%.

The strain of *B. microti* was kindly donated by Lise Gern (University of Neuchatel, Neuchatel, Switzerland). It was isolated from a bank vole (*Myodes glareolus*) in Central Switzerland and finally maintained by passages in female Swiss NMRI mice. The animals were sacrificed when the parasitemia reached 20%, and blood was collected by cardiac puncture. The course of infection was investigated using different infective doses. After intravenous inoculation with 2×10^7 *B. microti*-infected RBCs, mice typically showed a peak parasitemia of 70 to 80% by day 7 and a subsequent decrease to low or undetectable values by day 50.

Antibabesial agents. The diamidines were synthesized in the laboratories of David Boykin and Richard Tidwell as previously described (5, 11, 13). The syntheses of the unpublished compounds were achieved in an analogous manner. For *in vitro* studies, stocks of 10 mg/ml were prepared in dimethyl sulfoxide (DMSO) and subsequently diluted in RPMI cultivation medium, whereas for *in vivo* studies, compounds were dissolved in a 10% DMSO-water solution. Dimi-

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nazene aceturate (Berenil; Sigma, Steinheim, Germany), imidocarb dipropionate (Carbesia; Schering-Plough, kindly donated by Pierre Bonnemain), and atovaquone (GSK, Muenchenbuchsee, Switzerland) served as standard drugs. Standards were prepared as described above except for imidocarb dipropionate, a sterile solution that was diluted directly in RPMI or sterile deionized water.

Cytotoxicity determination. Cytotoxicity for L6 rat skeletal myoblasts was determined using the Alamar blue assay as described earlier (7, 21, 23).

***In vitro* growth-inhibitory assay.** Growth inhibition was determined by measuring the incorporation of radiolabeled [8-^{3}H]hypoxanthine (GE Healthcare, Amersham, United Kingdom) as described before (4). Twofold serial drug dilutions were prepared in 96-well microtiter plates in order to test seven drug concentrations to determine the 50% inhibitory concentrations (IC_{50}s). Duplicate wells received 100 μl of drug dilution and 100 μl of human RBC (2% parasitemia, 2.5% hematocrit). Controls consisted of infected RBCs without drug and noninfected RBCs. Plates were incubated at 37°C in a 4% CO_2, 3% O_2, 93% N_2 atmosphere for 48 h. Then 50 μl [8-^{3}H]hypoxanthine was added (0.5 μCi/well), and plates were incubated for another 24 h. Cells were then harvested on glass fiber filters with a cell harvester (Betaplate; Wallac PerkinElmer, Switzerland), the incorporated radioactivity was counted in a liquid scintillation counter (Betaplate; Wallac PerkinElmer, Switzerland), and IC_{50}s were calculated.

***In vivo* drug susceptibility test.** Female Swiss NMRI mice (18 to 20 g; RCC, Switzerland) were used for *in vivo* drug tests. On day 0, groups of three mice each were inoculated intravenously with 2×10^7 *B. microti*-infected RBCs. Diamidine compounds and standard drugs were administered subcutaneously or orally (atovaquone) in intervals of 4, 24, 48, and 72 h postinfection. Doses ranged from 50 to 12.5 mg/kg of body weight in a volume of 10 ml/kg. All experiments included a group of untreated controls. Tail blood was collected at least twice a week starting on day 7. Blood smears were stained with Giemsa, and parasitemia was determined microscopically with a detection limit of 1 parasite in 10,000 erythrocytes. A compound was defined as curative if no parasites were detectable up to day 60.

RESULTS

Different chemical classes of diamidines were screened for activity against *B. divergens in vitro* and *B. microti in vivo* and compared to the standard drugs diminazene aceturate, imidocarb dipropionate, and atovaquone. The results are summarized in Tables 1 and 2.

***In vitro* activity against *B. divergens*.** The dicationic molecules demonstrated a high selectivity for *B. divergens* compared to their cytotoxicity for mammalian cells, which was tested using L6 rat myoblast cells in an Alamar blue assay (7, 21, 23). Of the 214 diamidine compounds, 80 showed excellent IC_{50}s below 20 ng/ml, comparable to the *in vitro* activities of the standard antibabesial agents, but were up to 10 times more active than those agents. The best inhibitory effects on the growth of the cultured parasites were demonstrated by the benzimidiazoles, diphenyl furans, and indoles (Table 1). It is noteworthy that almost exclusively compounds of the class of the benzimidazoles also showed high *in vitro* activity against both *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense* (data not presented).

***In vivo* activity against *B. microti*.** NMRI mice infected with 2×10^7 parasitized mouse RBCs typically developed a peak parasitemia of 70 to 80% by day 7 postinfection followed by a second smaller peak of 20 to 25% around day 15. Subsequently, the parasitemia decreased to low and undetectable values (Fig. 1). The standard drugs diminazene at 25 mg/kg and imidocarb at 12.5 mg/kg administered on four consecutive days did not cure the mice but resulted in a delayed parasitemia peak of approximately 50% around day 23 followed by a decrease to 5% or less by day 30 postinfection. Most of the compounds tested *in vivo* were potent enough to cause rapid suppression and initial clearance of parasites from the blood by day 10 at a dosage of 25 mg/kg subcutaneously for the first 4 days. Failure to cure resulted in recrudescence by day 30, and peak parasitemia was generally seen 5 to 10 days after the relapse.

The best antibabesial properties were exhibited by the terphenyls, benzimidazoles, diphenyl furans, and pentamidine and its analogues as well as a diaryldiamidine (Table 2).

Three terphenyls provided cures of 2/3 (23DAP055, 19DAP085) or 3/3 (19DAP025) mice at a dosage of 25 mg/kg administered for 4 days. At a lower dose of 12.5 mg/kg, 19DAP025 still showed excellent activity and yielded a 3/3 cure.

The tested benzimidazoles were either very efficient in eliminating the parasites or could not reduce the parasite load significantly. DB818 and 6KXR030 cured 3/3 mice at a dosage of 25 mg/kg subcutaneously for 4 days. Administration of 12.5 mg/kg for 4 days resulted in a 2/3 cure for 6KXR030; however, in the case of DB818, all mice showed recrudescence around day 20 postinfection. DB942 and DB921 provided 100% suppression on day 10 but did not suppress parasitemia after day 18.

The diphenyl furan DB75, also known as furamidine, had excellent antibabesial activity when given subcutaneously. Four doses at 25 mg/kg cured all mice; at 20 mg/kg, 2/3 mice were cured, and at 12.5 mg/kg, 1/3 was cured. Even at lower doses, DB75 suppressed parasitemia completely through day 13 (6.25 mg/kg) or reduced parasitemia by >99% by day 7 (3.125 mg/kg). Mice treated with other diphenyl furans (DB530, DB555, and DB930) showed no detectable parasitemia until 10 days after the last of four injections of 25 mg/kg, but then parasites gradually reemerged.

Pentamidine is well known for its broad-spectrum antimicrobial activity, including antibabesial properties (24). In the series of pentamidine and pentamidine analogues, two compounds provided cures at 25 mg/kg: the pentamidine HCl 3SLT057 (1/3 cure) and the analogue 3KEG083 (3/3 cure).

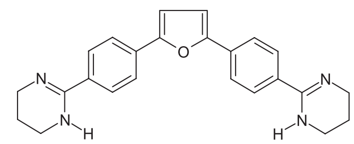
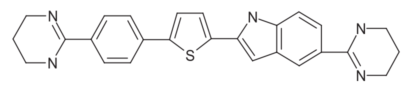
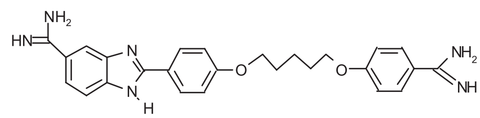
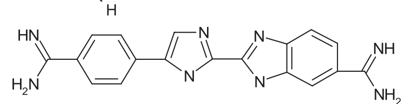
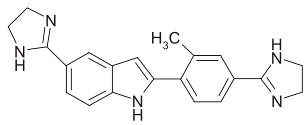
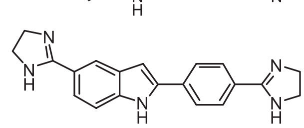
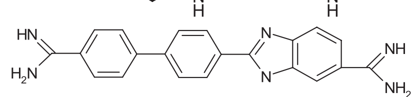
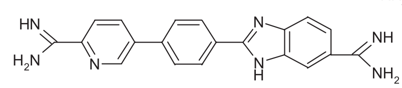
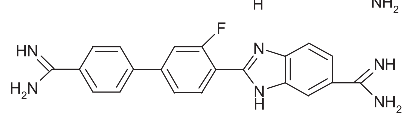
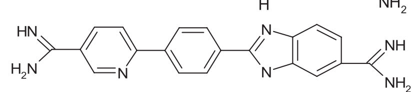
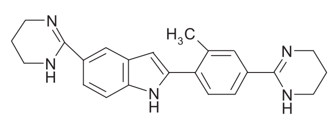
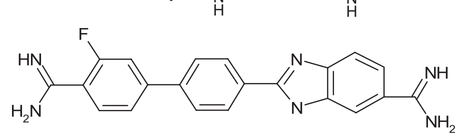
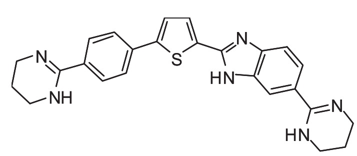
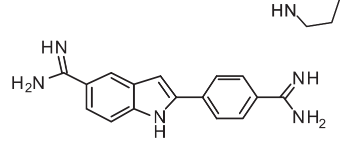
The isoxazoles and thiophenes did not show very good potency against *B. microti*. Although most of them caused a decrease in parasitemias to <1% by day 7, only one compound in each series could initially clear the parasites, and all mice had relapsed by day 17.

Further chemical groups tested only in small numbers included guanidino indenes, aza-furans, and biphenyls. All three groups seemed to bear considerable antibabesial potential. When administered subcutaneously on four consecutive days at 25 mg/kg, DB905A, DB820C, and DB986 cured 3/3, 2/3, and 1/3 mice, respectively.

In comparison with the diamidine compounds, the standard drugs were not particularly effective (Fig. 1). Even at a dosage of 50 mg/kg for 4 days, diminazene treatment resulted in a pronounced relapse. Atovaquone only reduced the parasite burden and slightly delayed the peak parasitemia. Only imidocarb dipropionate provided a 2/3 cure at a dosage of 25 mg/kg for 4 days.

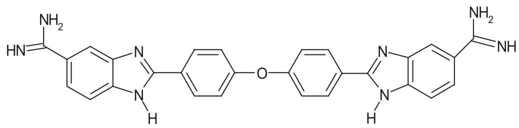
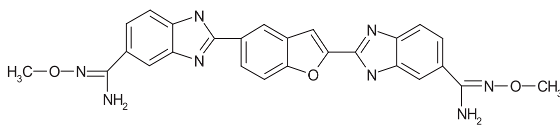
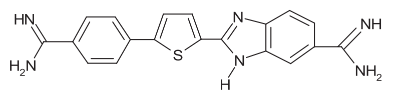
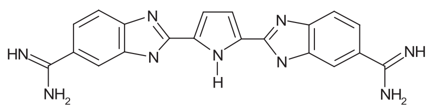
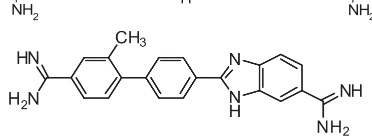
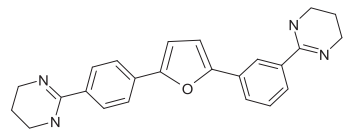
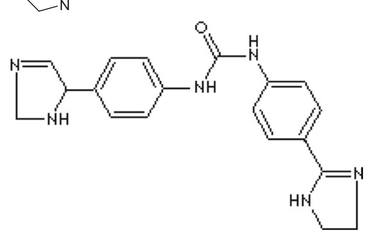
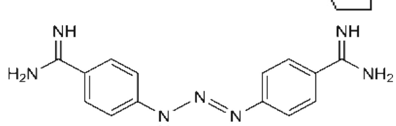
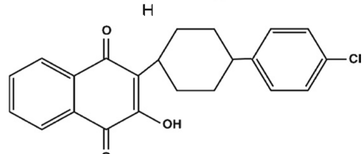
Correlation of *in vitro* activity and *in vivo* efficacy. Of the 25 compounds with low IC_{50}s (<20 ng/ml) tested *in vivo* against *B. microti*, 6 caused clearance of the parasites in one or more mice. A comparable number (7/30) of compounds with IC_{50}s in the middle range (20 to 85 ng/ml) could also eliminate *B. microti* from the blood.

TABLE 1. *In vitro* antibabesial and cytotoxic activities of diamidines

Compound (reference)	Structure	Chemical group	Mol wt	IC_{50} (μg/ml)[a] *B. divergens* strain 1903	IC_{50} (μg/ml)[a] *B. divergens* strain 4201	IC_{50} (μg/ml)[a] L6 cells
DB 0103 (5)		Diphenyl furan	457.00	0.0015	0.0017	>82.27
DB 1193[b]		Diaryl diamidine	553.00	0.0016	0.0026	19.2
48–702		Benzimidazole	654.64	0.0017	0.0018	26.7
DB 0940 (1)		Benzimidazole	583.40	0.0020	0.0021	65.5
DB 1191[b]		Indole	425.35	0.0021	0.0020	6.8
DB 1172[b]		Indole	402.30	0.0022	0.0021	1.8
DB 0921 (11)		Benzimidazole	566.00	0.0023	0.0027	9.6
DB 1250 (11)		Benzimidazole	581.80	0.0025	0.0020	15.07
DB 1236 (11)		Benzimidazole	575.00	0.0027	0.0034	17.71
DB 0922 (11)		Benzimidazole	541.00	0.0030	0.0035	13.8
DB 1190[b]		Indole	489.4	0.0030	0.0039	64.6
DB 0942 (11)		Benzimidazole	575.90	0.0035	0.0026	12.6
DB 1197[b]		Benzimidazole	581.50	0.0037	0.0054	42.7
DB 1171 (27)		Indole	354.75	0.0042	0.0038	0.6

Continued on following page

TABLE 1—*Continued*

Compound (reference)	Structure	Chemical group	Mol wt	IC50 (μg/ml)[a]		
				B. divergens strain 1903	*B. divergens* strain 4201	L6 cells
40–278		Benzimidazole	677.42	0.0048	0.0046	57.1
DB 0755[b]		Benzimidazole	636.10	0.0049	0.0051	13.2
DB 0818 (19)		Benzimidazole	474.00	0.0049	0.0051	10.6
DB 0192 (6)		Benzimidazole	546.80	0.0052	0.0159	>90
DB 0988 (11)		Benzimidazole	553.00	0.0054	0.0058	14.6
DB 0558 (3)		Diphenyl furan	474.50	0.0055	0.0050	87.1
Imidocarb		Standard drug	348.4	0.0106	0.0112	ND
Diminazene (diaceturate)		Standard drug	515.5	0.0158	0.0149	ND
Atovaquone		Standard drug	366.8	0.0091	0.0087	ND

[a] The IC_{50}s are mean values for at least two replicates. ND, not done.
[b] Not published.

DISCUSSION

The results presented in this study demonstrate that a variety of diamidines possess *in vitro* and *in vivo* antibabesial activities equal or superior to those of the existing drugs (diminazene aceturate, imidocarb dipropionate, and atovaquone).

The *in vitro* data with IC_{50}s of less than 10 ng/ml reflect the great antiprotozoal potential of the dications that had already been found against other protozoan parasites. A clear correlation between *in vitro* activities of the diamidines against *B. divergens*, *P. falciparum*, and *T. brucei rhodesiense*, however, was not evident. A fourth of the tested compounds showed IC_{50}s that were very similar to those of *P. falciparum*, and another fourth showed IC_{50}s that were very similar to those of *T. brucei rhodesiense*. Nevertheless, only a very limited number demonstrated comparable activity against all three parasites

TABLE 2. Activities of diamidine compounds and standard drugs against *B. microti* in NMRI mice and *B. divergens in vitro*

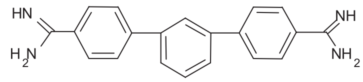
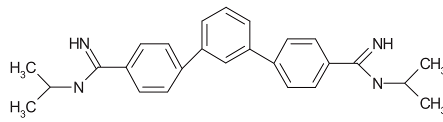
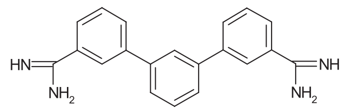
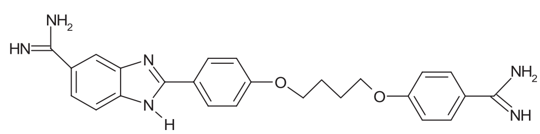
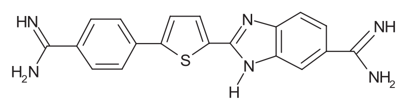
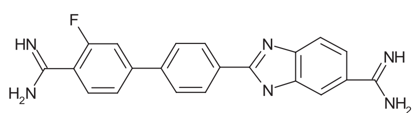
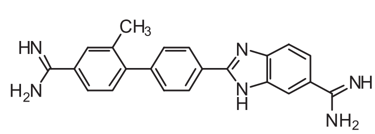
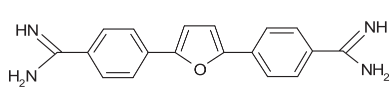
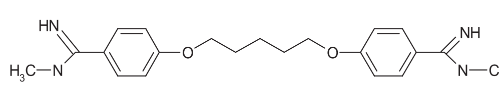
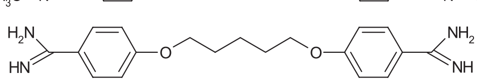
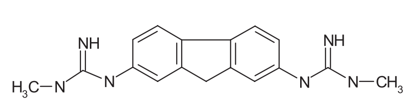
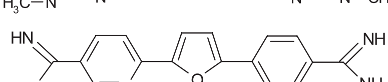
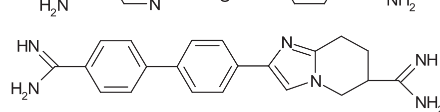
Chemical group	Compound (reference)	Structure	Dosage[a] (mg/kg)	*B. microti* cures[b]	IC_{50} (μg/ml)[c]	
					B. divergens strain 1903	*B. divergens* strain 4201
Terphenyls	19DAP025		25, 12.5	3/3, 3/3	0.0331	0.0318
	23DAP055		25	2/3	0.0633	0.0717
	19DAP085		25	2/3	0.0457	0.0446
Benzimidazoles	6KXR030		25, 12.5	3/3, 2/3	0.0081	0.0096
	DB818 (19)		25	3/3	0.0049	0.0051
	DB942 (11)		50	2/2	0.0035	0.0026
	DB988 (11)		50	1/2	0.0054	0.0058
Diphenyl furan	DB75 (5)		25, 20, 15, 12.5	3/3, 2/3, 1/3, 1/3	0.0112	0.0109
Pentamidine/ analogues	3KEG083		25	3/3	0.0101	0.0108
	3SLT057		25	1/3	0.0329	0.0377
Guanidino indene	DB905A (3)		25	3/3	0.0289	0.0314
Aza-furan	DB820C (13)		25	2/3	0.0410	0.0413
Biphenyl	DB986 (12)		25	1/3	0.0844	0.0903

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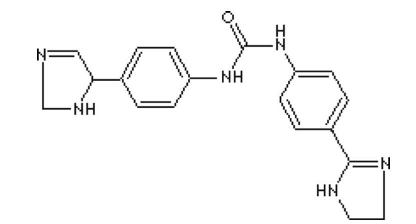
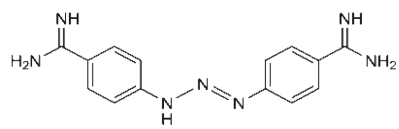
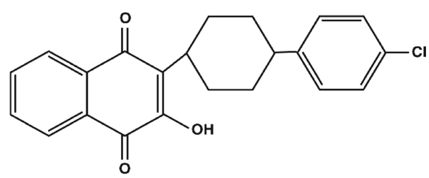
TABLE 2—*Continued*

Chemical group	Compound (reference)	Structure	Dosage[a] (mg/kg)	*B. microti* cures[b]	IC$_{50}$ (μg/ml) *B. divergens* strain 1903	IC$_{50}$ (μg/ml) *B. divergens* strain 4201
Standards	Imidocarb		25, 12.5	2/3, 0/3	0.0106	0.0112
	Diminazene		50	0/0	0.0158	0.0149
	Atovaquone		25	0/0	0.0091	0.0087

[a] All doses were given subcutaneously for 4 days.
[b] Results are given as number of cured animals/number of infected animals.
[c] The IC$_{50}$s are mean values for at least two replicates.

(data not shown). Interestingly, most of those compounds belonged to the benzimidazoles. This result could suggest that these parasites have common transporters which recognize the amidino-benzimidazole motif.

In the *B. microti* mouse model, the test compounds were considerably more active than the standard drugs. Diminazene aceturate and imidocarb dipropionate are used for treatment and prophylaxis of bovine, equine, canine, and ovine babesiosis in the field, usually as an intramuscular or subcutaneous injection of 3 to 5 mg/kg. In our *B. microti* mouse model, diminazene could not provide a cure at a 10-fold-higher dosage. In previous studies, where different antiprotozoal drugs had been screened against *B. microti* in Mongolian gerbils (24) and hamsters (20), diminazene was one of the most active drugs tested. Imidocarb, though more effective than diminazene, yielded only 2/3 cures at 25 mg/kg for 4 days, while several of the diamidines provided 3/3 cures. To our knowledge, there are no similar studies published where the efficacy of imidocarb was tested in *B. microti* mouse models, which is probably due to the fact that imidocarb is not licensed for human use. With atovaquone we expected to see a higher efficacy, since the drug had been reported to be very effective in *B. microti*-infected hamsters (10, 31) as well as in Mongolian gerbils infected with *B. microti* (9) or *B. divergens* (22). It was suggested, though, that *B. divergens* might be more sensitive than *B. microti*. In comparing our results with previous ones, it has to be considered that we used a different animal species and different treatment regimens. While in our study diminazene was administered at 50 mg/kg on four consecutive days, the Mongolian gerbils were treated at 20 mg/kg over a period of 14 days to achieve a complete eradication of the infection (24). Hughes and Oz administered atovaquone at doses up to 300 mg/kg over 2 weeks to hamsters and reported survival at day 24, but parasites were not cleared at all doses. In a further study in hamsters where atovaquone was used as monotherapy, recrudescences with the emergence of resistant organisms were described. Resistance could be prevented by the addition of

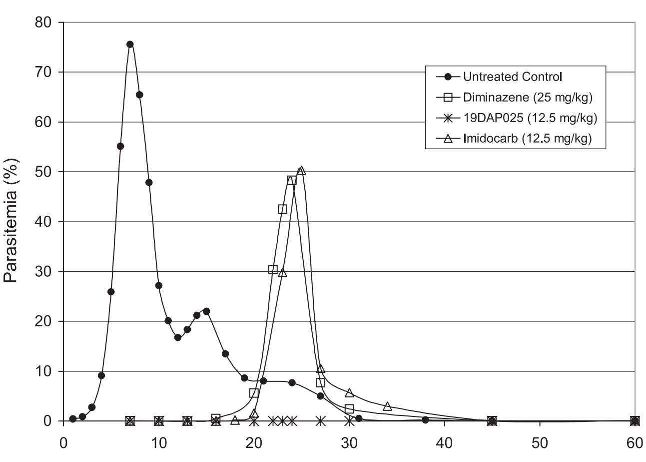


FIG. 1. Parasitemia in mice infected with *B. microti* in an untreated control group and after treatment with the standard drugs diminazene (25 mg/kg) and imidocarb (12.5 mg/kg) and with diamidine 19DAP025 (12.5 mg/kg). The standard drugs led to a delayed parasitemia of shorter duration than that for the untreated control, while the diamidine-treated mice were cured.

azithromycin (31). The combination of atovaquone and azithromycin is also used for the treatment of human babesiosis (17, 30). However, unlike the situation with *P. falciparum* (16), there has been no evidence of treatment failure due to resistant strains of *B. microti* (18) in humans. Nevertheless, for further *in vivo* experiments in our *B. microti* mouse model, combination treatments could provide valuable information.

The IC_{50} values of the *in vivo* active diamidines varied from 3.5 ng/ml to >60 ng/ml. This finding suggests that a low IC_{50} should not be the only selection criterion for *in vivo* testing, as pharmacological parameters are as important as the antiparasitic activity. The ability of IC_{50}s to predict *in vivo* activity is problematic, and thresholds should not be set too low when compounds are being selected for *in vivo* trials.

In conclusion, the data presented in this report indicate the potential of dicationic aromatic molecules as antibabesial agents. It is evident that further studies of structure-activity relationships, mode of action, toxicity, and *in vivo* efficacy of these compounds are needed before a clinical candidate can be selected.

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REFERENCES

1. **Batista-Parra, A.** 2003. Synthesis of 5-[2-(5-amidinobenzimidazoyl)-2-(4-amidinophenyl)furan hydrochloride to test a DNA minor groove dimer binding model. Diss. Abstr. Int. B **63:**5843.
2. **Bell, C. A., et al.** 1990. Structure-activity relationship of analogs of pentamidine against *Plasmodium falciparum* and *Leishmania mexicana amazonensis*. Antimicrob. Agents Chemother. **34:**1381–1386.
3. **Boykin, D. W., et al.** 1995. Dicationic diarylfurans as anti-*Pneumocystis carinii* agents. J. Med. Chem. **38:**912.
4. **Brasseur, P., S. Lecoublet, N. Kapel, L. Favennec, and J. J. Ballet.** 1998. In vitro evaluation of drug susceptibilities of *Babesia divergens* isolates. Antimicrob. Agents Chemother. **42:**818–820.
5. **Das, B. P., and D. W. Boykin.** 1977. Synthesis and antiprotozoal activity of 2,5-bis-(4 guanylphenyl)furans. J. Med. Chem. **20:**531.
6. **Del Poeta, M., et al.** 1998. Structure-in vitro activity relationships of pentamidine analogues and dicationic substituted bis-benzamidazoles as new antifungal agents. Antimicrob. Agents Chemother. **42:**2495–2510.
7. **Ganapaty, S., P. Steve Thomas, G. Karagianis, P. G. Waterman, and R. Brun.** 2006. Antiprotozoal and cytotoxic naphthalene derivatives from *Diospyros assimilis*. Phytochemistry **67**(17)**:**1950–1956.
8. **Granström, M.** 1997. Tick-borne zoonoses in Europe. Clin. Microbiol. Infect. **3:**156–169.
9. **Gray, J. S., and M. Pudney.** 1999. Activity of atovaquone against *Babesia microti* in the Mongolian gerbil, *Meriones unguiculatus*. J. Parasitol. **85**(4)**:** 723–728.
10. **Hughes, W. T., and H. S. Oz.** 1995. Successful prevention and treatment of babesiosis with atovaquone. J. Infect. Dis. **172**(4)**:**1042–1046.
11. **Ismail, M. A., et al.** 2005. Dicationic near-linear biphenyl benzimidazole derivatives as DNA-targeted antiprotozoal agents. Bioorg. Med. Chem. **13:** 6718–6726.
12. **Ismail, M. A., et al.** 2004. Novel dicationic imidazo[1,2-a]pyridines and 5,6,7,8-tetrahydro-imidazo[1,2-a]pyridines as antiprotozoal agents. J. Med. Chem. **47:**3658–3664.
13. **Ismail, M. A., R. Brun, F. Tanious, W. D. Wilson, and D. W. Boykin.** 2003. Synthesis and anti-protozoal activity of aza-analogs of furamidine. J. Med. Chem. **46:**4761–4769.
14. **Kjemtrup, A. M., and P. A. Conrad.** 2000. Human babesiosis: an emerging tick-borne disease. Int. J. Parasitol. **30:**1323–1337.
15. **Kocken, C. H., et al.** 2006. *Plasmodium vivax*: in vitro susceptibility of blood stages to synthetic trioxolane compounds and the diamidine DB75. Exp. Parasitol. **113:**197–200.
16. **Korsinczky, M., et al.** 2000. Mutations in *Plasmodium falciparum* cytochrome *b* that are associated with atovaquone resistance are located at a putative drug-binding site. Antimicrob. Agents Chemother. **44:**2100–2108.
17. **Krause, P. J., T. Lepore, V. K. Sikand, J. Gadbaw, Jr., et al.** 2000. Atovaquone and azithromycin for the treatment of babesiosis. N. Engl. J. Med. **343**(20)**:**1454–1458.
18. **Krause, P. J., et al.** 2008. Persistent and relapsing babesiosis in immunocompromised patients. Clin. Infect. Dis. **46**(3)**:**370–376.
19. **Mallena, S., et al.** 2004. A thiophene based diamidine forms a "super" AT binding minor groove agent. J. Am. Chem. Soc. **126:**13659–13669.
20. **Miller, L. H., F. A. Neva, and F. Gill.** 1978. Failure of chloroquine in human babesiosis (*Babesia microti*): case report and chemotherapeutic trials in hamsters. Ann. Intern. Med. **88:**200–202.
21. **Page, C., M. Page, and C. Noel.** 1993. Fluorometric assay for cytotoxicity measurements in vitro. Int. J. Oncol. **3:**473–476.
22. **Pudney, M., and J. S. Gray.** 1997. Therapeutic efficacy of atovaquone against the bovine intraerythrocytic parasite, *Babesia divergens*. J. Parasitol. **83**(2)**:** 307–310.
23. **Räz, B., M. Iten, Y. Grether-Buhler, R. Kaminsky, and R. Brun.** 1997. The Alamar Blue assay to determine drug sensitivity of African trpyanosomes (*T.b. rhodesiense* and *T.b. gambiense*) in vitro. Acta Trop. **68**(2)**:**139–147.
24. **Ruebush, T. K., P. G. Contacos, and E. A. Steck.** 1980. Chemotherapy of *Babesia microti* infections in Mongolian jirds. Antimicrob. Agents Chemother. **18:**289–291.
25. **Stead, A. M., et al.** 2001. Diamidine compounds: selective uptake and targeting in *Plasmodium falciparum*. Mol. Pharmacol. **59:**1298–1306.
26. **Tidwell, R. R., and D. W. Boykin.** 2003. Dicationic DNA minor groove binders as antimicrobial agents, p. 414–460. *In* M. Demeunynck, C. Bailly, and W. D. Wilson (ed.), DNA and RNA binders: from small molecules to drugs, vol. 2. Wiley-VHC Verlag GmbH, Weinheim, Germany.
27. **Tidwell, R. R., et al.** 1978. Diarylamidine derivatives with one or both of the aryl moieties consisting of an indole or indole-like ring. Inhibitors of arginine-specific esteroproteases. J. Med. Chem. **21:**613.
28. **Vannier, E., B. E. Gewurz, and P. J. Krause.** 2008. Human babesiosis. Infect. Dis. Clin. North Am. **22:**469–488.
29. **Vial, H. J., and A. Gorenflot.** 2006. Chemotherapy against babesiosis. Vet. Parasitol. **138:**147–160.
30. **Weiss, L. M.** 2002. Babesiosis in humans: a treatment review. Expert Opin. Pharmacother. **3:**1109–1115.
31. **Wittner, M., J. Ledermann, H. B. Tanowitz, G. S. Rosenbaum, and L. M. Weiss.** 1996. Atovaquone in the treatment of *Babesia microti* infections in hamsters. Am. J. Trop. Med. Hyg. **55**(2)**:**219–222.